The Bovine Corneal Opacity and Permeability (BCOP) Assay

Presentation to:
ICCVAM Expert Panel

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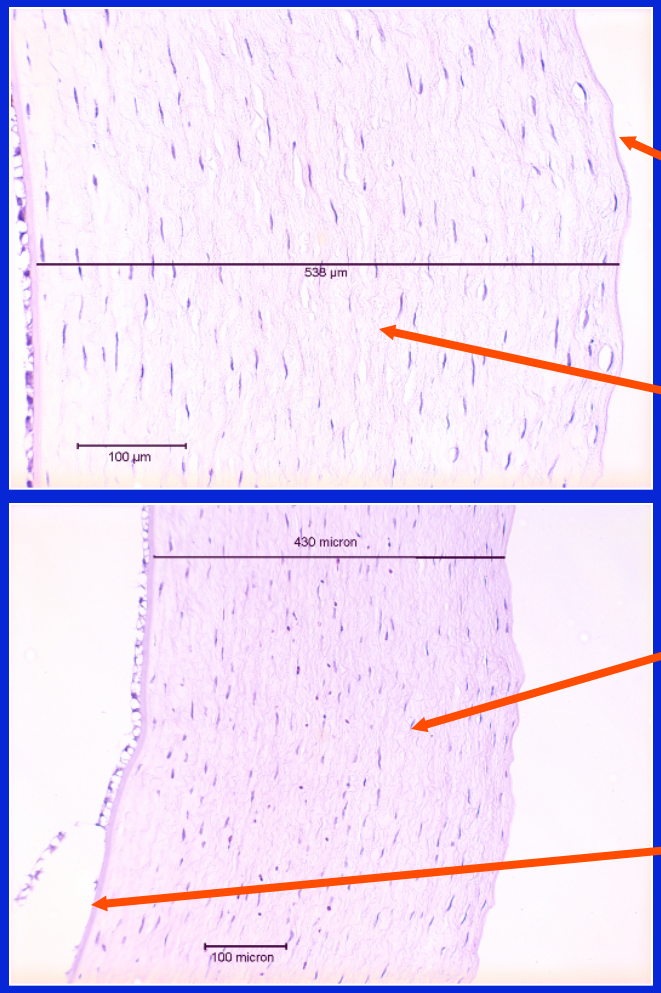
With special appreciation to Drs. Pierre Gautheron and Joseph Sina, Merck Research

Corneal burn caused by alkali

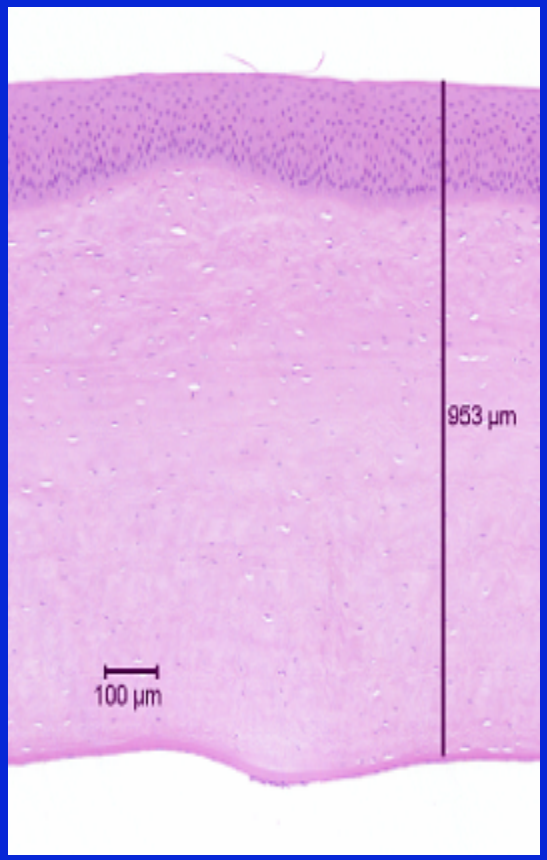
Corneal Lesions Associated with Eye Irritation In Vivo



- Epithelial cell loss
  - Partial or full thickness loss over some fraction of the cornea
- Stromal swelling (change in hydration state)(reversible) or denaturation (poorly reversible)
- Death of keratocytes
  - Depth of injury/loss associated with increased severity of the lesion (and initiation of inflammation)
- Endothelial cell loss (cells do not regenerate in humans)

Micrographs from Cuellar et al, 2003

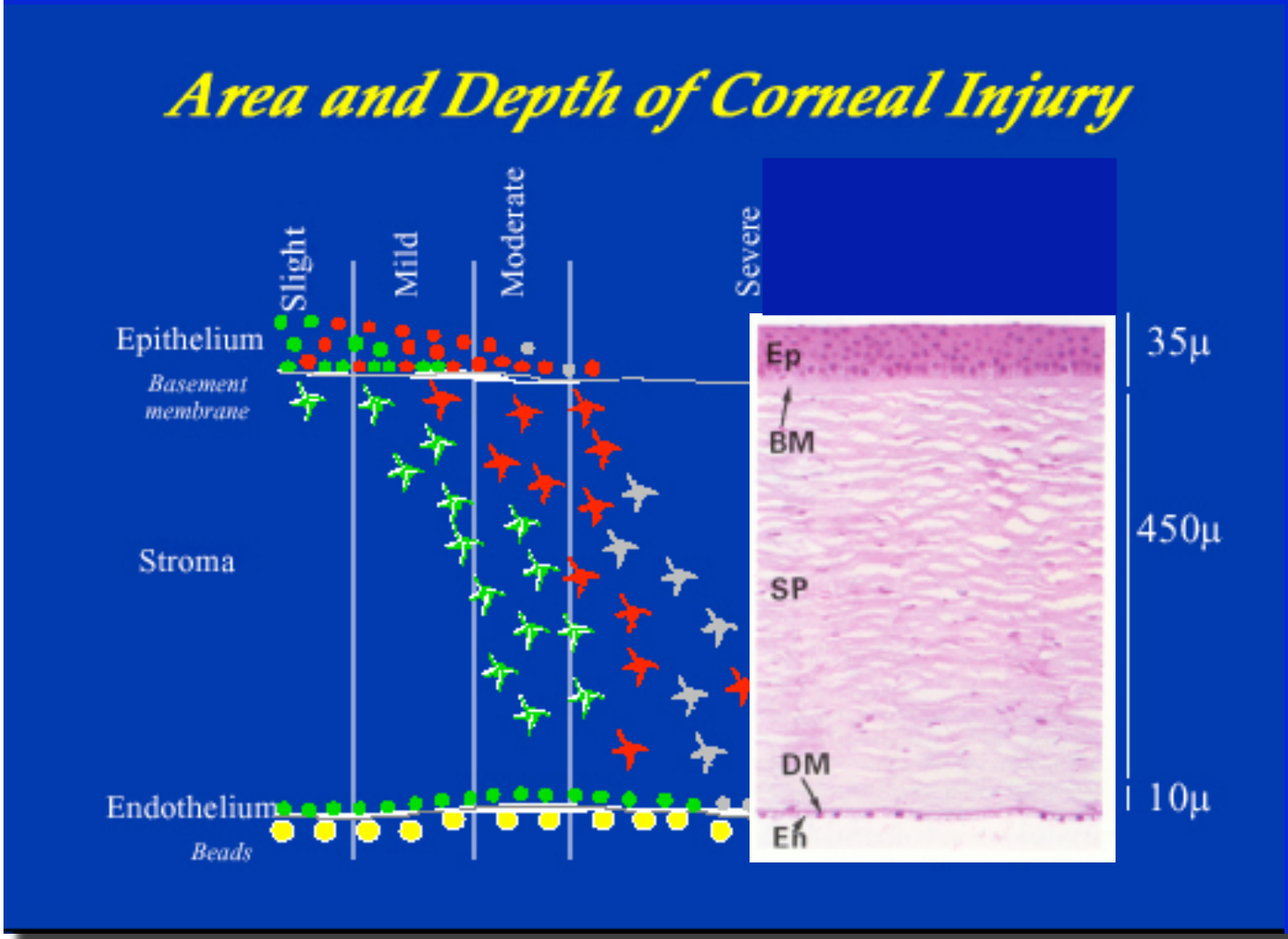
Common Modes of Chemical Action in Eye Irritation



- Membrane lysis
  - Surface active agents solubilize membrane lipids
  - Organic solvents extract lipids
- Protein Coagulation/Denaturation
  - Acids and certain solvents
- Saponification
  - Alkali (often progressive)
- Alkylation, Oxidative Damage to Macromolecules
  - Reactive materials such as bleaches and peroxides

Depth of Injury Model

Depth of injury is predictive of the degree and duration of injury



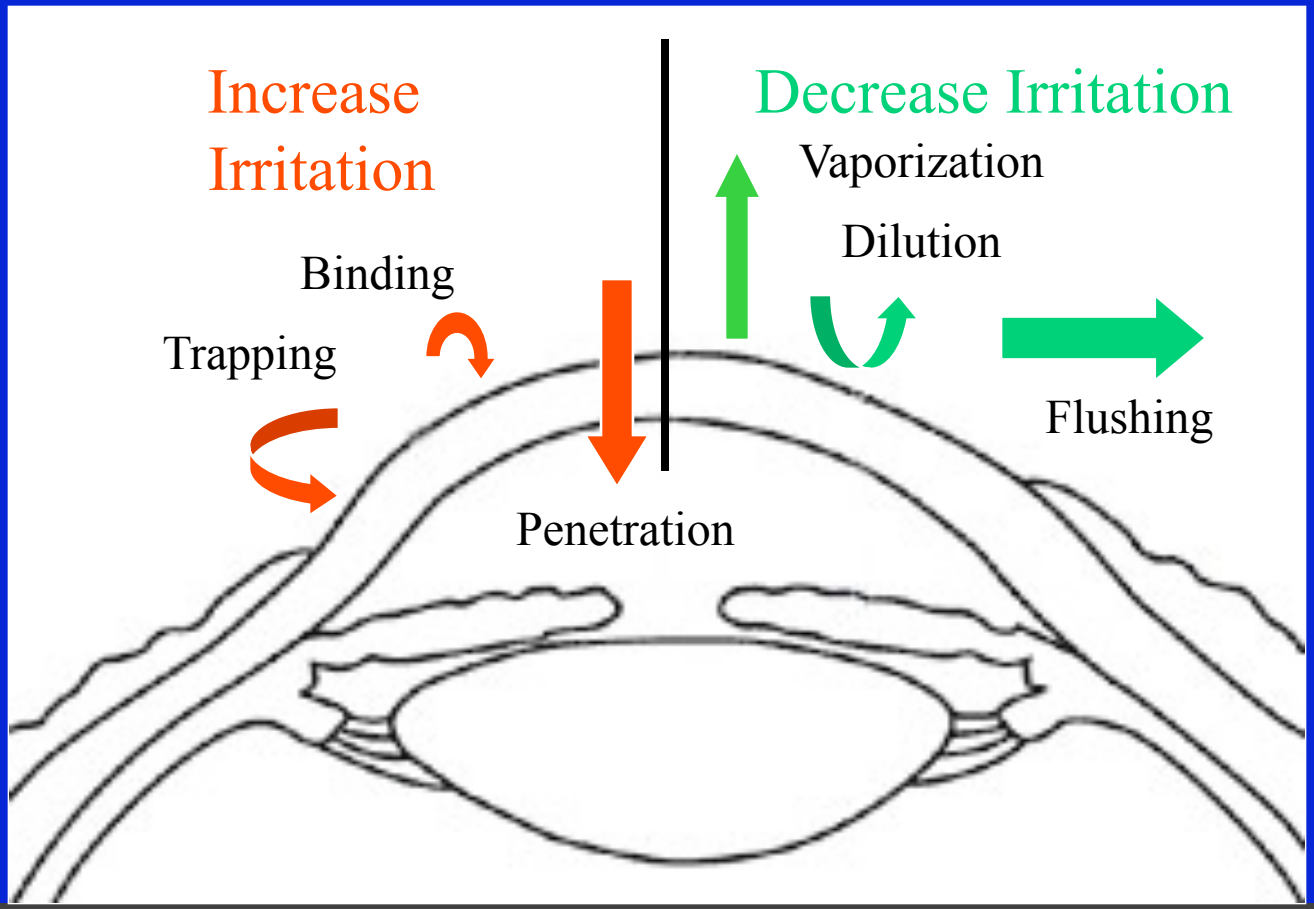
Based on the work of J. Maurer and J. Jester, taken from R. Osborne

Using the Ex Vivo Cornea

- For an in vitro assay to address a wide range of chemistries and degrees of irritancy potential, it should:
  - Provide the appropriate cellular and structural targets
  - Allow determination of depth of injury
  - Respond to the various modes of action of irritants on the cornea
- The bovine cornea appears to fulfill these requirements

Factors Impacting Exposure in the Eye

Exposure = volume x concentration x time

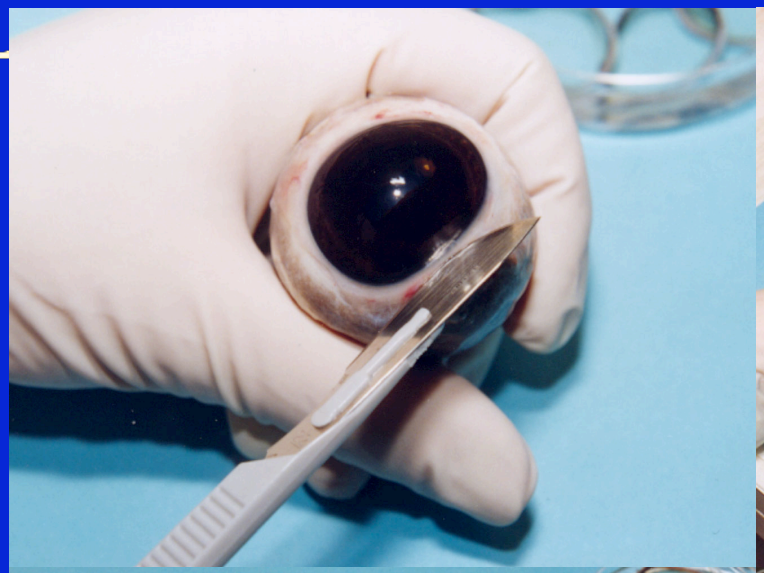


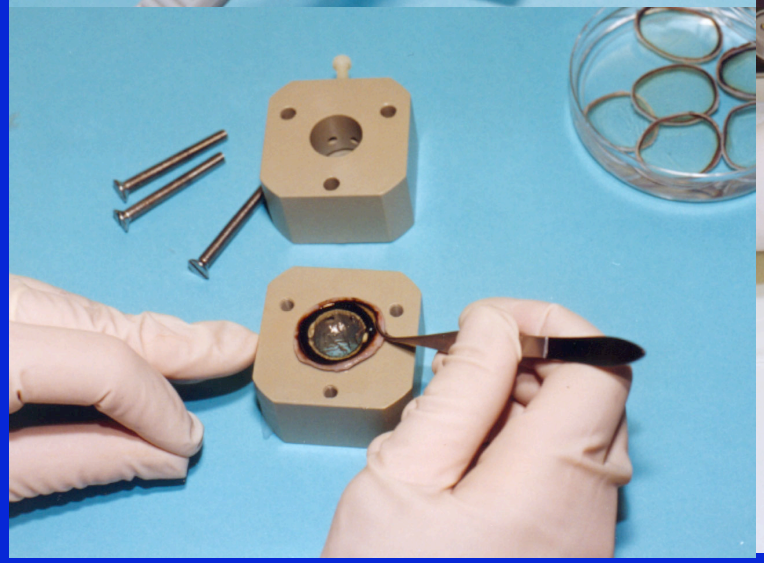
- Rapid binding and/or penetration will favor increased irritation potential.
- Therefore, the formulation may alter the irritation potential of actives as well as the "inerts".
- These "variables" add to the difficulty of modeling exposures in vitro.

Development of In Vitro Tests for Determining Eye Irritation Potential

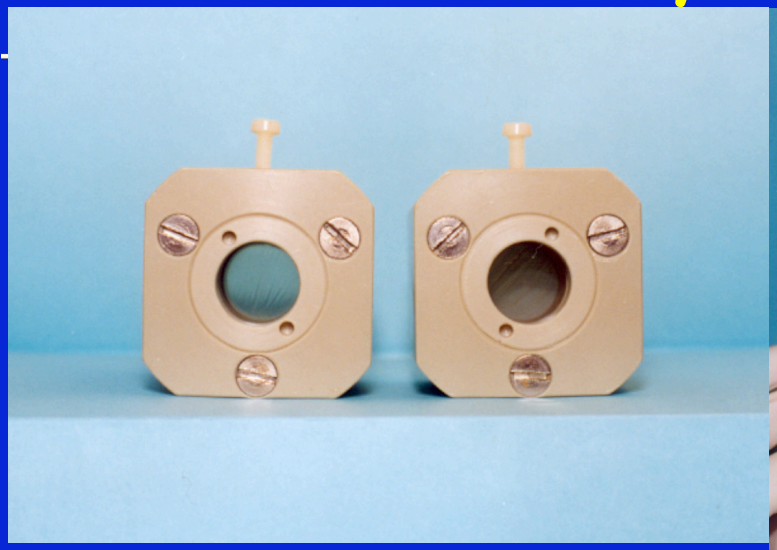
- Focus on control of the independent variables
  - Target tissue (test system)
  - Exposure
    - Define and control volume, concentration, and time of exposure
  - Endpoints
    - Measure the initial events after exposure that are predictive of subsequent macroscopic changes
    - Machine scored (except histology)
  - Concurrent controls and benchmark materials
    - Used to track consistency and establish acceptance criteria
  - Multi-laboratory studies to demonstrate performance over time and across laboratories

BCOP Assay (Summary 1)





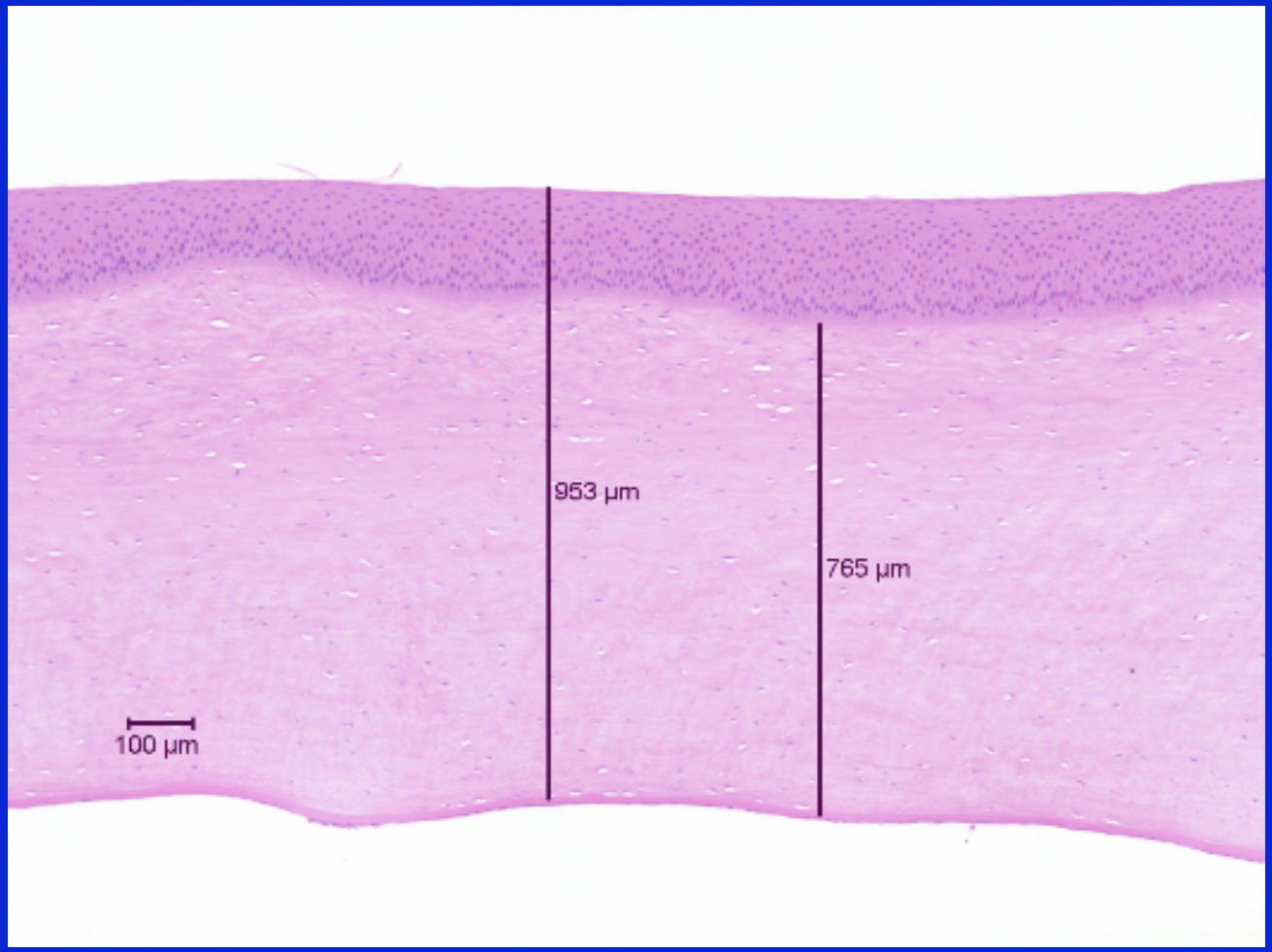
BCOP Assay (Summary 2)



Photographs provided by

Dr. Joe Sina, Merck Research

Corneal Structure (Bovine)



Epithelium

Stroma

Descemet's Membrane and Endothelium

Key Features of the BCOP: Exposure

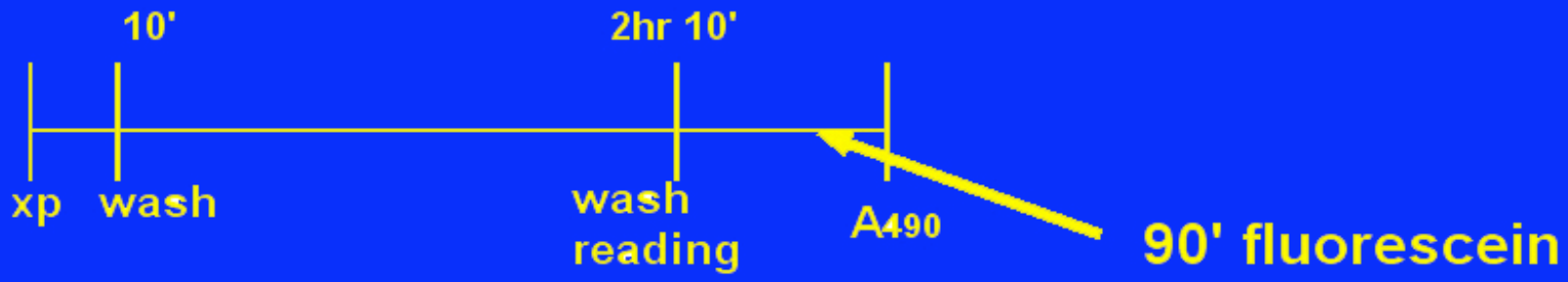
- Viable corneas maintained in organ culture
- Control over the exposure concentration
- Control over the exposure time at the specified concentration
- Exposure over the whole corneal surface
- Control over the post-exposure (expression) period

Key Features of the BCOP: Endpoints for Assessing Tissue Injury

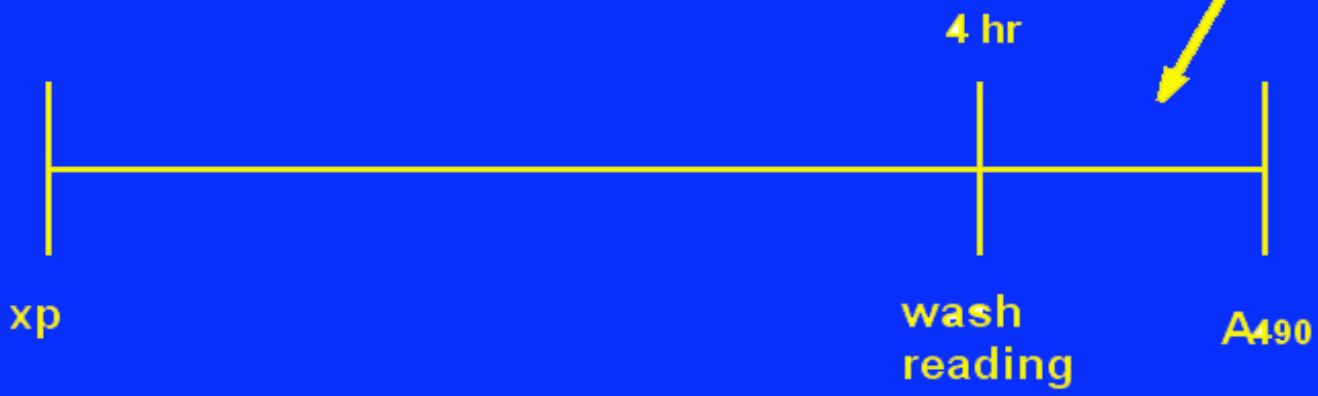
- **Quantitative** change in light passage (opacity)
- **Quantitative** change in the barrier integrity of the epithelium as measured by fluorescein penetration through the cornea
- Additional endpoints
  - Histology - quite common in our laboratory
  - Corneal hydration
  - Endothelial cell layer integrity
- In Vitro Score = Opacity + 15 x OD_{490}
- Certain chemicals do not induce direct opacity and so only the permeability score is used (e.g. anionic and nonionic surfactants).
- Positive Controls always used. Benchmark materials used when ever possible.

Bovine Corneal Opacity-Permeability Assay

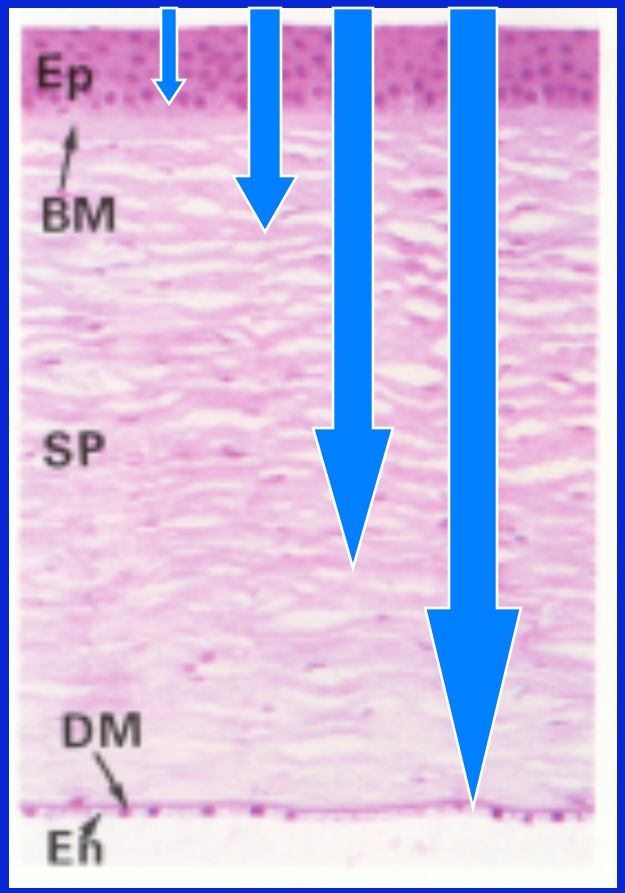
liquids (100%)



solids (200 mg/ml)



Specialized Applications of the BCOP Assay



- Specifically modified exposure and post-exposure times are used to address certain chemical classes, expected consumer exposure scenarios or to enhance comparisons across a product class.
- As opposed to the rabbit test, the BCOP can be modified to assist in risk assessment.

Using the BCOP in the Real World

- Test materials acting through membrane lysis, denaturation and/or saponification generally produce opacity and/or permeability changes that allow the determination of degree of irritancy without extended post-exposure incubation or histology. Reactive chemicals require extended incubation and histology to rule out occult damage. Understanding the relative depth of injury, through histology, can be useful for all modes of action.
- Experience with the EC/HO chemicals has shown that when the mode of action is not known, the most conservative approach is to address the reactive chemistry mode by using extended post-exposure incubation and histology. The more rapid changes will also be detected with this approach.

A Continuum of Sensitivity